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Identification and Properties of Chlamydial Polypeptides That Bind Eucaryotic Cell Surface Components

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An electroblotting technique was used to identify proteins of *Chlamydia* that bound surface-radioiodinated and Triton X-100-solubilized HeLa cell extracts. Two proteins, with apparent molecular masses of 18 and 32 kilodaltons (kDa), that bound HeLa cell surface components were identified on *Chlamydia trachomatis* L2 elementary bodies (EBs). Radioiodinated heparin, which disrupts chlamydial association with cultured cells, was also bound by these proteins. These two proteins were found on EBs but were absent or were present in reduced amounts on the noninfectious reticulate bodies. All *C. trachomatis* strains tested displayed two such proteins, although the apparent molecular weight of the larger protein varied with serotype in correlation with biotype and the disease that it caused. Two *Chlamydia psittaci* strains examined displayed only a single binding protein in the range of 17 to 19 kDa. All of the binding proteins stained intensely and distinctively on silver-stained sodium dodecyl sulfate-polyacrylamide gels and displayed an unusual sensitivity to reducing agents. The 32-kDa protein was not seen and did not bind ^{125}I-labeled HeLa cell components if the EBs were solubilized in the presence of 2-mercaptoethanol. The 32-kDa protein was not affected by dithiothreitol, however. Similar to the effect of 2-mercaptoethanol, the 32-kDa protein was not visualized after treatment of EBs with the protease inhibitors tosyl-phenylalanine chloromethyl ketone (TPCK) or tosyl-lysine chloromethyl ketone (TLCK). TPCK and TLCK also abolished infectivity as did the alkylating agents *N*-ethylmaleimide and iodoacetamide, yet the latter two agents did not affect the appearance of the 32-kDa protein. These proteins were not detected in immunoblots with either rabbit antisera to *C. trachomatis* L2 EBs or by serum from a patient with lymphogranuloma venereum. The role of these proteins in the interaction of chlamydiae with host cells is not clear, but the binding of eucaryotic cell surface components and heparin, presence only during the infectious stage of the life cycle, variation between serotypes in correlation with disease, and sensitivity to reducing agents or protease inhibitors, collectively, suggest a role for these proteins in parasite-host interactions.

Chlamydia species are procaryotic obligate intracellular parasites of eucaryotes. Characteristics that distinguish these bacteria from other obligate intracellular parasites are (i) a complex life cycle that includes an infectious extracellular cell type, the elementary body (EB), and a noninfectious, intracellular, and replicating cell type, the reticulate body (RB) (5, 14, 35); (ii) disulfide bonding of outer membrane proteins as a mechanism of maintaining structural stability (4, 18, 19, 30) in the absence of peptidoglycan (2, 16, 27, 40); and (iii) replication of the parasite within phagosomes apparently modified by the bacteria to inhibit fusion with lysosomes (5, 14, 35).

There are two species of *Chlamydia*, *C. trachomatis* and *C. psittaci*. The two species share a number of biological properties but differ antigenically (10) and exhibit only about 10% DNA homology (21, 31). There are 15 serotypes within the species *C. trachomatis* (35). These may be grouped into two biovars, the strains that cause lymphogranuloma venereum (LGV strains; serotypes L1, L2, and L3) and the non-LGV-causing strains (non-LGV strains; serotypes A to K) (35). The LGV strains are more invasive pathogens, while the infections caused by serotypes A through K are usually localized to mucous membranes. The non-LGV strains can be subdivided on the basis of the diseases that they cause; serotypes A, B, Ba, and C are associated with endemic blinding trachoma while the remaining serotypes, D to K, are usually associated with urogenital infections or inclusion conjunctivitis, although more serious infections may occur (35).

In additon to their epidemiology, the *C. trachomatis* biovars differ in biological properties observed in vitro. LGV and non-LGV strains differ in their associations with host cells. Attachment and inclusion formation by non-LGV strains is greatly enhanced by polycations in the medium or centrifugation of inocula onto cells (24), while such treatments have little effect on LGV association with host cells. The mechanism(s) of chlamydial attachment and internalization is not yet clear. It has been suggested recently that chlamydiae, like some viruses and polypeptide hormones, may use a constitutive cellular process such as receptor-mediated endocytosis to gain entry into eucaryotic cells (38). However, in other recent studies it has been found that endosomes containing chlamydia were not coated with clathrin (43). The internalization of chlamydia, therefore, was felt to differ from the receptor-mediated endocytosis of viruses and hormones into clathrin-coated vesicles (43).

The nature of the ligand on the EB surface that recognizes and interacts with the host cell surface is also unknown. I used an electroblotting procedure to demonstrate the association of radiolabeled eucarytoic cell components with separated chlamydial proteins. The chlamydial polypeptides identified in this manner exhibited several intriguing biological properties. These polypeptides were associated only with the infectious form of the parasite and varied in subunit molecular weight among chlamydial serotypes. Variability in

size of these polypeptides directly correlated with the virulence properties of the organism.

(A preliminary account of this work was presented at the 84th Annual Meeting of the American Society for Microbiology, St. Louis, Mo., March 1984).

MATERIALS AND METHODS

Organisms. *C. trachomatis* strains LGV-434, serotype L2; LGV-404, serotype L3; B/Tw-5/OT, serotype B; D/UW-3/CX, serotype D; G/UW-57/CX, serotype G; H/UW-4/CX, serotype H; and I/UW-12/UR, serotype I, and *C. psittaci* strains that cause meningopneumonitis and guinea pig inclusion conjunctivitis were grown and purified from HeLa 229 or L-929 cells as described previously (9). Intrinsic radiolabeling of EBs with ^{14}C-labeled amino acids or [^{35}S]cysteine was also as described previously (11).

PAGE. Polyacrylamide gel electrophoresis (PAGE) was carried out as described by Laemmli (25), except that both the stacking gel and resolving gel contained 2 mM EDTA. Immunoblotting procedures have been described previously (18).

Binding of eucaryotic cell components. A monolayer of HeLa 229 cells was rinsed once with Hanks balanced salt solution and scraped from a 150-cm^2 flask with a rubber policeman. The cells were washed twice with Hanks balanced salt solution by low-speed centrifugation (250 × *g*, 10 min) and suspended in Hanks balanced salt solution and surface radioiodinated by the lactoperoxidase procedure (29). The ^{125}I-labeled cells were suspended in 50 mM sodium phosphate–150 mM NaCl–phosphate-buffered saline (PBS; pH 7.4)–1% (vol/vol) Triton X-100 and incubated with constant mixing for 2 h at 37°C. The suspension was then centrifuged at 12,000 rpm for 10 min in a Beckman Microfuge 12 (Beckman Instruments, Inc., Fullerton, Calif.), and the supernatant was saved.

Chlamydial EBs were suspended in 2% sodium dodecyl sulfate (SDS)–10% glycerol–62.5 mM Tris hydrochloride (pH 6.8) and immediately solubilized by immersion in a boiling water bath for 10 min. For some experiments, 4% 2-mercaptoethanol (2-ME) was included in this mixture. The solubilized EBs were subjected to SDS-PAGE and electrophoretically transferred to nitrocellulose paper (HAHY; Millipore Corp., Bedford, Mass.) at 27 V/cm and 1.0 A for 2 h at 17°C in 25 mM sodium phosphate (pH 7.2). Blocking was with PBS–0.05% Tween 20 or with PBS plus 3% bovine serum albumin. The nitrocellulose sheets to which chlamydial polypeptides were transferred were placed in plastic bags and incubated for 2 h in the presence of 2 × 10^6 cpm of ^{125}I-labeled HeLa cell extracts diluted in PBS–0.05% Tween 20. This dilution resulted in a final Triton X-100 concentration of about 0.01 to 0.04%. The nitrocellulose was then removed from the bag, washed once with PBS–0.05% Tween 20 and several times with water, dried, and subjected to autoradiography. In some cases the nitrocellulose sheet was stained with amido black 10B (18) before autoradiography.

Infectivity determinations. The effect of various protease inhibitors and sulfhydryl active agents on infectivity was determined as follows. *C. trachomatis* L2 EBs were suspended in 250 mM sucrose–10 mM sodium phosphate–5 mM glutamate, (SPG; pH 7.2). Soy trypsin inhibitor, chymostatin, dithiothreitol (DTT), iodoacetamide, and *N*-ethylmaleimide were made up in sterile distilled water at 10 times the final concentrations tested (Table 1) and diluted 1:10 in the EB suspension. The remaining inhibitors were solubilized in absolute ethanol and diluted 1:40 in the EB suspension to give a final concentration of 2 mM. An equal volume of absolute ethanol was added to control suspensions. This amount of ethanol had no effect on infectivity. The suspensions were incubated for 2 h at 37°C and then pelleted and washed once with SPG. The EBs were then suspended and diluted in SPG prior to the inoculation of HeLa cell monolayers on cover slips for determination of inclusion-forming units as described previously (15, 18).

RESULTS

Binding of HeLa cell components by chlamydial proteins. Chlamydial whole cell lysates after SDS-PAGE and electrophoretic transfer to nitrocellulose paper, were incubated in the presence of ^{125}I-surface-labeled and Triton X-100-solubilized HeLa cell extracts. Two polypeptides of about 18 and 32 kilodaltons (kDa) bound solubilized HeLa cell surface components (Fig. 1). The 32-kDa polypeptide was not seen in SDS-PAGE profiles and did not bind ^{125}I-labeled HeLa cell surface proteins if the EBs were solubilized for electrophoresis in the presence of 2-ME. Reduction by DTT followed by alkylation or alkylation alone before solubilization had no effect on the migration of these polypeptides or their ability to bind HeLa surface components.

Binding of [^{125}I]heparin. Heparin has been shown to interfere with chlamydial association with host cells (6, 23) and,

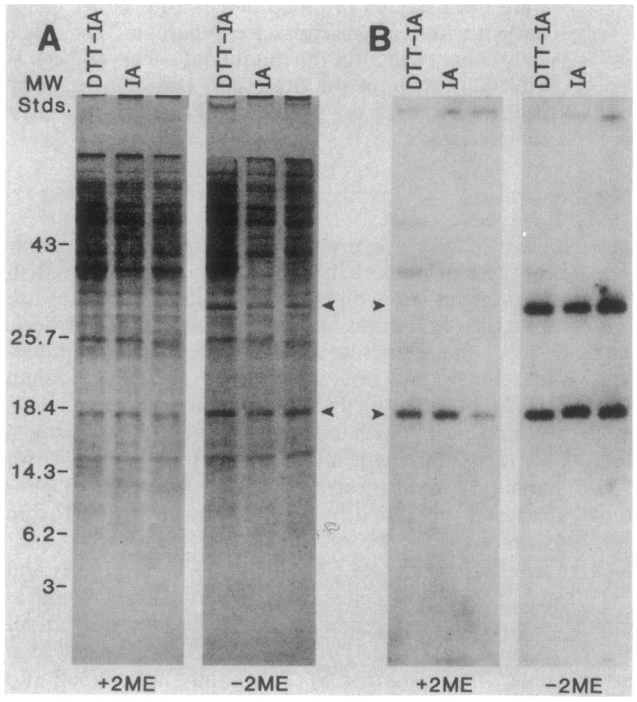

FIG. 1. Binding of ^{125}-labeled eucaryotic cell surface components by chlamydial proteins immobilized on nitrocellulose filters. *C. trachomatis* L2 EBs were treated before solubilization with 10 mM DTT followed by treatment with 20 mM iodoacetamide (DTT–IA), iodoacetamide alone (IA), or no treatment. Duplicates were then solubilized in the presence or absence of 2-ME for SDS-PAGE. (A) Coomassie brilliant blue-stained profiles of 15% polyacrylamide gels. MW Stds., Molecular weight standards, in thousands. (B) Autoradiograms of parallel gels after electrophoretic transfer to nitrocellulose and incubation in the presence of surface-radiolabeled and Triton X-100-solubilized HeLa cells.

more recently, to displace bound but not internalized chlamydia from the eucaryotic cell surface (T. Hackstadt, S. F. Hayes, and H. D. Caldwell, Abstr. Annu. Meet, Am. Soc. Microbiol. 1985, D5, p. 55). We examined the ability of these proteins to bind [^{125}I]heparin (Fig. 2). Both the 18- and 32-kDa proteins bound heparin. These polypeptides, however, did not bind to heparin-agarose columns after solubilization with Sarkosyl or SDS (data not shown).

Comparison of EBs with RBs. Polypeptide profiles of purified EBs and RBs were compared by SDS-PAGE with Coomassie brilliant blue staining (Fig. 3). The two polypeptides which bound HeLa surface proteins and heparin were present on the EB but absent or, in the case of the 18-kDa protein, reduced in amount on the RBs. Again the 32-kDa polypeptide was not observed if the samples were dissociated in the presence of 2-ME.

[^{35}S]cysteine labeling. The two components of interest stained distinctively by the silver-staining procedure described by Tsai and Frasch (41). However, the significance of differences in color and intensity of ammoniacal silver-stained proteins in polyacrylamide gels is not clear. To address the question of whether these chlamydial components were indeed protein, or whether they contained protein, EBs were intrinsically labeled in vivo with [^{35}S]cysteine; and the susceptibility of the solubilized [^{35}S]EB proteins to proteinase K was examined (Fig. 4). The 18- and 32-kDa polypeptides both contained cysteine, and both were digested by proteinase K after solubilization and denaturation by SDS. The labeling of these components by [^{35}S]cysteine and their susceptibility to protease suggests that they are at least in part proteinaceous.

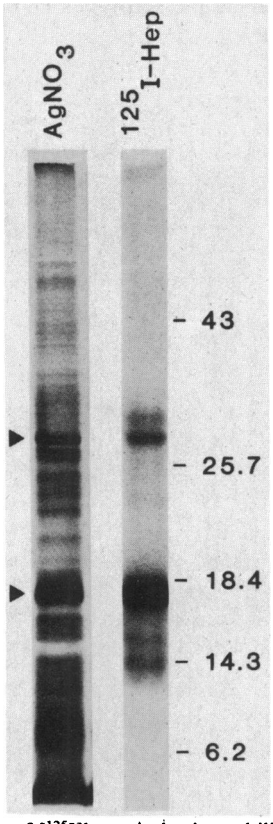

FIG. 2. Binding of [^{125}I]heparin by immobilized chlamydial proteins. The left lane is a silver-stained 12.5% polyacrylamide gel profile of *C. trachomatis* L2 EBs solubilized in the absence of 2-ME, while the right lane is an autoradiogram of a parallel sample after transfer to nitrocellulose and binding of [^{125}I]heparin (^{125}I-Hep). Arrowheads indicate the position of proteins that bind HeLa cell surface constituents and heparin. Numbers to the right of the gels are molecular weights, in thousands. These proteins stain intensely by the silver-staining procedure of Tsai and Frasch (41), often with the bands displaying a distinct golden center. The EBs display a number of other less prominant polypeptides that also bind heparin.

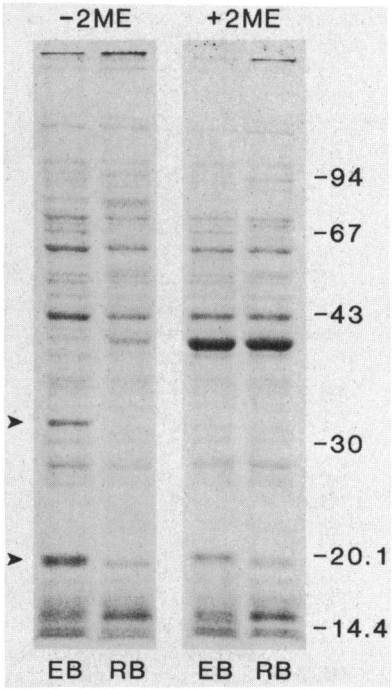

FIG. 3. Coomassie brilliant blue-stained 12.5% polyacrylamide gel profile of *C. trachomatis* L2 EBs and RBs solubilized in the presence (+) or absence (−) of 2-ME. Free sulfhydryl groups were blocked by alkylation before solubilization to minimize mixed disulfide formation during solubilization. This treatment did not seem to affect the appearance of the proteins in question. The polypeptides indicated by arrowheads bind HeLa cell components. Numbers to the right of the gels are molecular weights, in thousands.

Effects of protease inhibitors. I examined the effects of a variety of protease inhibitors on chlamydial infectivity (Table 1). Of those examined, only tosyl-lysine chloromethyl ketone (TLCK) and tosyl-phenylalanine chloromethyl ketone (TPCK), inhibitors of trypsin and chymotrypsin, respectively, abolished chlamydial infectivity. The effects of these agents on the 18- and 32-kDa proteins was examined on silver-stained polyacrylamide gels (Fig. 5). The 32-kDa polypeptide was not evident in the gel profile if the EBs were solubilized in the presence of TPCK or TLCK. The alkylating agents iodoacetamide and *N*-ethylmaleimide, which were also lethal to chlamydiae, did not affect the appearance of the 32-kDa protein on SDS-PAGE.

The sensitivity of the 32-kDa polypeptide to 2-ME is puzzling. The protein is not apparent in the SDS-PAGE profile of EBs solubilized in the presence of 2-ME whether identified by Rm and Coomassie brilliant blue or silver staining, autoradiograms of intrinsically radiolabeled EBs, or binding of iodinated HeLa cell surface components. When the intrinsically ^{14}C-amino acid-labeled 32-kDa protein was cut from a gel run without 2-ME solubilization and resolubil-

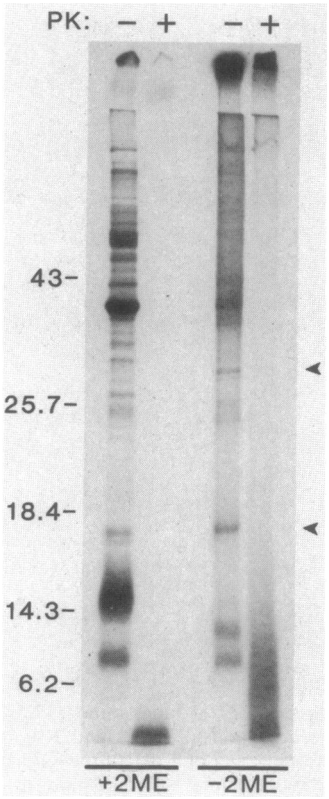

FIG. 4. Autoradiogam of intrinsically [^{35}S]cysteine-labeled *C. trachomatis* L2 EBs solubilized for SDS-PAGE in the presence or absence of 2-ME. Solubilized EBs were treated (+) or not treated (−) with proteinase K (PK; 0.5 mg/ml) for 1 h at 56°C before electrophoresis. Arrowheads indicate the position of chlamydial proteins that bind HeLa cell constituents. Numbers to the left of the gels are molecular weights, in thousands.

ized and run in the presence of 2-ME, a diffuse radioemitting pattern resulted over the molecular mass range of 25 to 32 kDa. However, the 32-kDa protein was not affected by DTT. Besides being a reducing agent, 2-ME can also act as a chelating agent for divalent cations (28). It seems unlikely that this activity is responsible for these observations since 5 mM EDTA or EGTA [ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid] in the solubilization buffer had no effect on the 32-kDa proteins (data not shown).

Surface exposure? If the 32- and 18-kDa proteins are involved in the interaction of chlamydia with host cells, one might predict that they would be exposed on the EB outer surface. Intact *C. trachomatis* LGV-434 EBs were therefore surface iodinated by the Iodogen procedure (13) and subjected to SDS-PAGE and autoradiography. The 32-kDa polypeptide was not labeled by this radioiodination procedure, although the 18-kDa peptide was weakly radiolabeled (Fig. 6).

Antigenicity of the 18- and 32-kDa polypeptides. Neither of the two polypeptides reacted by immunoblotting with hyperimmune antisera prepared in rabbits against Formalin-killed (or viable; data not shown) *C. trachomatis* LGV-434 EBs or with a human LGV convalescent serum (Fig. 7).

Identification of binding proteins on other chlamydial strains. There are 15 serotypes of *C. trachomatis* (35). These 15 serotypes may be subdivided into three biovars on the basis of biological properties and diseases caused. I compared representative serotypes from each of the three *C. trachomatis* biovars and two *C. psittaci* strains (Fig. 8). All three of the *C. trachomatis* biovars exhibited two polypeptides that bound ^{125}I-labeled HeLa cell surface components, while the two *C. psittaci* strains showed only a single binding protein. The binding protein of the LGV strains, serotypes L2 and L3, migrated to positions of about 18 and 32 kDa, while the equivalent polypeptides of the strains that cause urogenital infections, serotypes D, G, and H (as well

TABLE 1. Effects of protease inhibitors on *C. trachomatis* infectivity

Inhibitor (conc.)	IFUs (mean ± SEM, ×10^6)[a]
Control	2.41 ± 0.34
Tosyl-arginine methyl ester (2 mM)	2.18 ± 0.09
TLCK (2 mM)	0
TPCK (2 mM)	0
Diazo-norleucine methyl ester (2 mM)	1.91 ± 0.10
Phenylmethyl sulfonyl fluoride (2 mM)	2.50 ± 0.06
Soy trypsin inhibitor (100 μg/ml)	2.92 ± 0.12
Chymostatin (100 μg/ml)	2.92 ± 0.15
DTT (5 mM)	0.35 ± 0.04
Iodoacetamide (5 mM)	0
N-Ethylmaleimide (5 mM)	0

[a] IFUs, Inclusion-forming units.

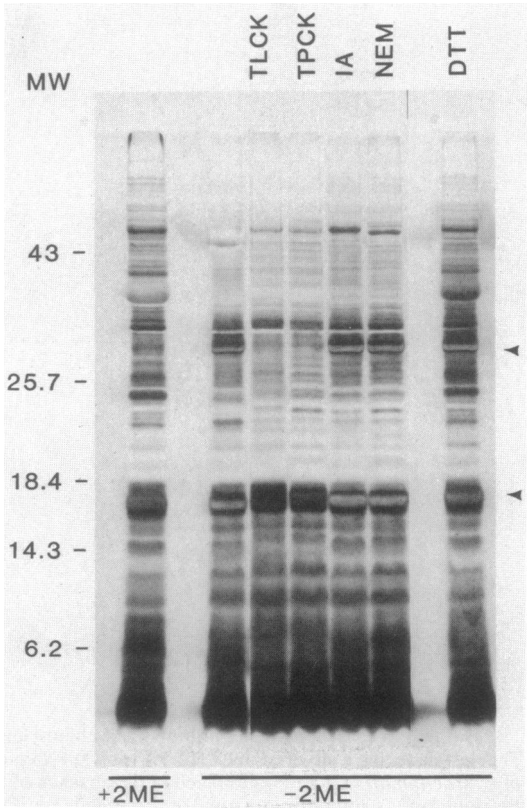

FIG. 5. Silver-stained 12.5% polyacrylamide gel profile of *C. trachomatis* L2 EBs solubilized in the presence (+) or absence (−) of 2-ME, or without 2-ME but with the following: 2 mM TLCK, 2 mM TPCK, 5 mM iodoacetamide (IA), 5 mM *N*-ethylmaleimide (NEM), or 5 mM DTT. Arrowheads indicate the position of the proteins that bind HeLa cell surface components. Numbers to the left of the gels are molecular weights (MW), in thousands.

as I and J; data not shown), migrated as proteins of 18 and 27 kDa. A single endemic trachoma strain, serotype B, displayed binding proteins of about 18 and 23 kDa. In all *C. trachomatis* strains examined, the larger polypeptide varied in size among *C. trachomatis* biotypes and appeared to be present in reduced quantity in the stained gel profile if the EBs were solubilized in the presence of 2-ME (data not shown). In contrast to *C. trachomatis*, the *C. psittaci* strains that cause meningopneumonitis and guinea pig inclusion conjunctivitis displayed only a single polypeptide that bound ^{125}I-labeled HeLa cell extracts. This polypeptide differed slightly in apparent molecular mass between the two strains and differed from the *C. trachomatis* strains but migrated in the range of 17 to 19 kDa.

DISCUSSION

I have described here chlamydial polypeptides that exhibit a number of distinctive properties that, collectively, are suggestive of a biological role for these components as adhesins in chlamydial-host interaction. These polypeptides are present on the infectious EBs but not on the RBs; bind eucaryotic cell surface components and heparin; vary in apparent molecular weight in correlation with biovar, virulence, and disease; are affected by reducing conditions or protease inhibitors; and are apparently nonimmunogenic on intact EBs.

There are a number of stages in the chlamydial life cycle at which any function of these proteins might be exerted. A priori, the attachment and internalization of EBs would seem to be a likely step at which parasite components which bind eucaryotic cell surface proteins might function. However, the suggestion of a role for these components must take into account the observation that these proteins were poorly iodinated, if at all, on the EB surface. One possibility is that these polypeptides may not have exposed tyrosine, tryptophan, or histidine residues available for iodination. Another consideration is that these polypeptides may not be surface exposed until triggered by some close association with the host. The resistance of extracellular EBs to the effects of a number of proteases led us previously to consider that proteolytic cleavage of some inactive precursor on the EB surface might be required to expose active sites involved in attachment or penetration (17). Analogy was drawn to the FO protein of paramyxoviruses, of which proteolytic cleavage is required to activate the hemagglutinating and cell fusion activities of the cleavage product (36, 37). If proteolytic cleavage of some EB component were required for expression of infectivity, the protease involved could be of host or chlamydial origin. If, however, an endogenous chlamydial protease were involved, this hypothetical protease would be resistant to a number of serine protease inhibitors. Other classes of proteases may not be affected by these inhibitors.

The 32-kDa polypeptide was not seen on Coomassie brilliant blue or silver-stained polyacrylamide gels if the EBs were solubilized in the presence of the protease inhibitors TPCK or TLCK. TPCL and TLCK are specific inhibitors of trypsin and chymotrypsin, respectively, but chloromethyl

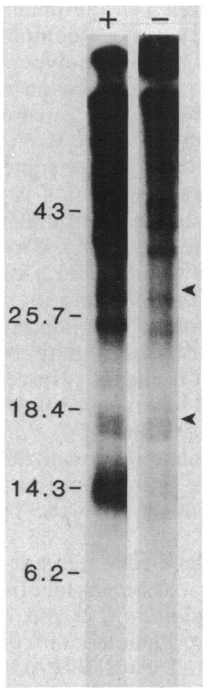

FIG. 6. Autoradiogram of *C. trachomatis* L2 EBs surface labeled by the Iodogen procedure (13) and subjected to SDS-PAGE after solubilization in the presence (+) or absence (−) of 2-ME. The position of the chlamydial binding proteins is indicated by arrowheads. The position of these bands was determined by overlaying the autoradiogram over the Coomassie brilliant blue-stained and dried gel. Numbers to the left of the gels are molecular weights, in thousands.

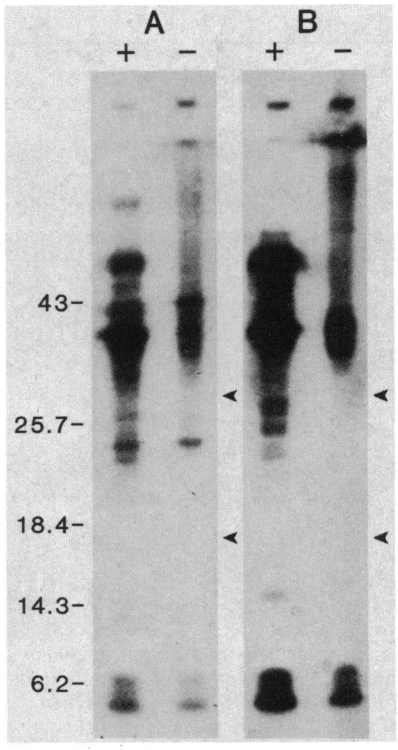

FIG. 7. Immunoblot of *C. trachomatis* L2 EBs solubilized in the presence (+) or absence (−) of 2-ME before SDS-PAGE. Antigenic polypeptides were detected with rabbit polyclonal antisera to *C. trachomatis* L2 EBs (A) or human LGV patient serum (B). Positions of the chlamydial binding proteins were localized by overlaying the amido black 10B-stained nitrocellulose sheet and are indicated by arrowheads. Numbers to the left of the gels are molecular weights, in thousands.

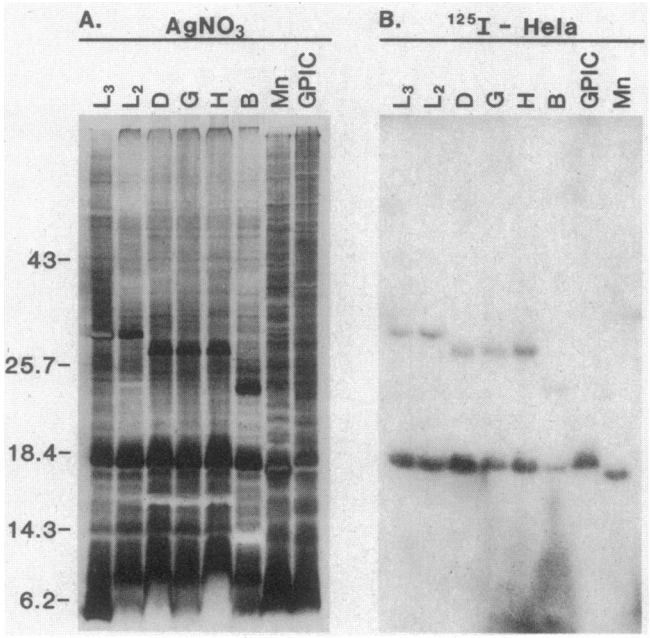

FIG. 8. Strain heterogeneity of the putative chlamydial adhesins. (A) A silver-stained gel profile of *C. trachomatis* serotypes L3, L2, D, G, H, and B, and *C. psittaci* strains that cause meningopneumonitis (Mn) and guinea pig inclusion conjunctivitis (GPIC). Samples were solubilized for electrophoresis without 2-ME. (B) A parallel gel transferred to nitrocellulose, and the potential adhesins were detected by adsorption of ^{125}I-labeled HeLa cell components (^{125}I-HeLa). Numbers to the left of the gels are molecular weights, in thousands.

ketones can also act nonspecifically as alkylating agents (32). However, the alkylating agents *N*-ethylmaleimide and iodoacetamide did not have this effect under identical conditions. It may be that this reflects some difference in solubility or other property that allows access of chloromethyl ketones, but not other alkylating agents, to essential sulfhydryl groups. It should also be remembered that the EBs are cells with a number of proteins and other components exposed on their surface. It is probably not surprising that inhibitors with different specificities might decrease infectivity by acting at different sites on the same or different proteins which may function independently or in concert to mediate the interactions of parasite and host.

The 18- and 32-kDa proteins share some properties, yet the precise relationship between the two is not yet understood. Several observations are inconsistent with the 32-kDa protein being a disulfide-linked dimer of the 18-kDa protein, including the following: (i) the apparent molecular mass of the larger band varied among serogroups while that of the 18-kDa protein varied little, (ii) re-electrophoresis of the 32-kDa protein after excision from polyacrylamide gels and solubilization with 2-ME did not give a fragment of 18 kDa (data not shown), (iii) the amount of the 18-kDa protein on SDS-PAGE did not obviously increase after 2-ME solubilization, and (iv) the effect was limited to 2-ME and not DTT. Differential effects of dithiols versus monothiols on enzyme activity have been described previously (26). I, too, have seen differential effects of 2-ME versus DTT on the 32-kDa protein as well as on the oxidation of glutamate by EBs in vitro (18). It is unclear, however, whether these differences are due to some effect on an enzymatic activity or surface structure in general.

The sensitivity of the binding activity of the 32-kDa protein to 2-ME cannot be adequately explained from the results presented here, but the fact that it occurs in an organism that depends on disulfide bonding for structural stability and therefore requires reduction or disulfide exchange for differentiation suggests a possible mechanism of regulating activity through disulfide interactions.

The polypeptides described here were found not to be reactive by immunoblot analysis or radioimmunoprecipitation with rabbit antisera to Formalin-killed EBs (data not shown). The rabbit antisera used here did not neutralize infectivity. In most cases, antisera produced in rabbits to EBs have neutralized poorly or not at all (8, 20, 33; H. D. Caldwell, personal communication; T. Hackstadt, unpublished data). Assigning a role for these proteins awaits the production of specific antibodies to address function. While the function of these proteins is still not known, it should be considered that it would be to the parasites advantage if proteins important in its interaction with the host were nonimmunogenic. Protection when it does occur is type specific (1, 7, 42). At least two surface proteins of *C. trachomatis* have been shown to have type-specific epitopes: the major outer membrane protein (12) and a 27- to 32-kDa protein described by Sacks et al. (34). The latter protein is close to the size of one of the proteins that I describe here. The polypeptides described here varied in apparent molecular weight with biovar or epidemiology, but antigenic or structural analysis of these proteins will be required to determine the extent of variation that occurs.

The ability of SDS and heat-denatured chlamydial polypeptides, after transfer to nitrocellulose, to bind HeLa cell surface proteins is surprising in itself. If these proteins do indeed have a function in the chlamydial life cycle and association with eucaryotic cell components is part of that function, the domains of these polypeptides which mediate interaction with host proteins remain active after denaturation by heat and SDS and transfer to nitrocellulose. Whether primary structure alone is enough to mediate the interactions seen here or whether some higher order structure remains after these treatments is unknown. An additional possibility is that these proteins might possess other moieties, carbohydrates or lipids, for example, that may function in the recognition by HeLa cell components. The nature of the interaction is therefore open to question. The observation that the polyanionic heparin also binds to these proteins suggests that charge may be involved. Indeed surface charge and hydrophobicity have been shown to vary among *C. trachomatis* serotypes (39). The variation of these binding proteins among groups of *C. trachomatis* that vary in biological properties make the localization of these proteins even more necessary. If these are surface exposed, they could conceivably contribute to the biological differences among strains.

Adsorption of radiolabeled, detergent-solubilized bacterial proteins to eucaryotic cells have identified binding proteins of *Mycoplasma pneumoniae* (22) and *Treponema pallidum* (3). The procedure described here differs in that the bacterial proteins were separated by SDS-PAGE and electroeluted onto nitrocellulose before incubation in the presence of radiolabeled, Triton X-100-solubilized eucaryotic cell components. Using this technique, I was successful in identifying two polypeptides that vary in quantity between the infectious and noninfectious forms of the life cycle and in apparent molecular weight between serotypes that differ in biological properties. Whether these proteins, indeed, represent chlamydial adhesins awaits more detailed analysis,

but they possess enough properties that correlate with biology and virulence to suggest that they are at least worthy of further study.

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